ETHIONINE INDUCED sRNA NEOMETHYLASE ACTIVITY

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Received March 4, 1968

Recent studies in this laboratory have been directed towards the methylation of sRNA using adult, embryonic, and neoplastic mouse liver cell preparations (Hancock, 1966; Hancock, 1967a; Hancock, 1967b; Hancock, et al., 1967). After characterizing the sRNA ethylase activity of mouse liver (manuscript submitted) investigations were initiated to detect any changes in sRNA ethylase activity of ethionine treated mice. Although the sRNA ethylase activities thus far are not remarkable, it was discovered during experiments analyzing rates of methylation that sRNA methylase activity had definitely increased in livers from ethionine treated mice. This paper will present evidence that new cytidylate, adenylate, and uridylate sRNA methylase activities arise in the liver cells of ethionine treated mice. Only data concerned with mice fed ethionine for six months will be presented here and a full report including three month data will appear elsewhere after the analysis of mice fed for nine months with an ethionine diet and completion of sRNA ethylase experiments.

MATERIALS AND METHODS

E. coli K12 sRNA was purchased from General Biochemicals, Inc., Chagrin Falls, Ohio. S-adenosyl-L-(C^{14} methyl) methionine was purchased from New England Nuclear, Boston, Mass., and unlabeled S-adenosyl-L--methionine from Sigma, Inc., St. Louis, Mo. The sRNA methylase activity

was assayed according to the method of Srinivasan and Borek (1963), and enzyme preparations were prepared as previously described (Hancock, 1967a) using male C3HeB/J mice. Ethionine treated mice received 0.5 per cent ethionine using a ground Old Guilford diet (11 per cent fat - 19 per cent protein) for six months.

RESULTS AND DISCUSSION

Experiments on the rate of sRNA methylation showed that the liver supernatant fraction from ethionine treated mice incorporated over two and a half times as many methyl groups as did control preparations (control: 40.23 ± 1.89 $\mu\mu$moles/hr/10 mg protein, ethionine treated: 117.97 ± 5.83 $\mu\mu$moles/hr/10 mg protein, $t = 12.52$, d.f. = 10, $P < 0.001$).

It can be seen in Table I that a greater extent of methylation, which demonstrates differences in the sites of methylation, is accomplished by sRNA methylases present in liver cells from ethionine fed mice than with control mice. This new activity, possibly by the induction of new enzymes (neomethylases) from the ethionine treated mice, is also more active upon

TABLE I.

The Increase in Extent of Methylation by Liver Enzymes from Ethionine Fed Mice. ($\mu\mu$moles methyl groups incorporated)

Liver Enzyme Preparation	*E. coli* sRNA	Incubation time		
		0.5 min	50 min	75 min
Control	-	2.59	33.71	34.00
Control	+	3.17	102.31	102.02
Ethionine treated	-	6.52	107.49	119.88
Ethionine treated	+	6.92	275.79	233.43

Reaction mixture contained: 100 μmoles tris pH 7.6, 30 μmoles $MgCl_2$, 50μmoles reduced glutathione, 0.5 μC of S-adenosyl-L-(C^{14} methyl)-methionine (3.12 mC/mmole), 100 μg of *E. coli* K12 sRNA as indicated, and 1 ml of 100,000 x g supernatant from male C3HeB/J mouse liver prepared according to Hancock (1967a) in a total volume of 2 ml., incubated for the indicated times at 37°C and assayed according to Srinivasan and Borek (1963).

mouse liver sRNA as demonstrated by the endogenous activity. Similar results are found using C57L/J liver as compared with BW7756 hepatoma preparations. The increase in the endogenous activity of the ethionine treated preparations is not believed to be due to an increase in amount of mouse liver hypomethyl sRNA. Since the extent of methylation by the control liver enzymes is 68.6 $\mu\mu$moles/100 μg E. coli sRNA as contrasted to 168.3 $\mu\mu$moles/100 μg E. coli sRNA by liver enzymes from ethionine treated mice, the increase in methylation of liver sRNA would not be unexpected. However, sRNA from ethionine fed and control mice will be isolated and used as substrate for methylation by mouse liver enzymes to examine the possibility of increased hypomethyl sRNA.

From Table II one can determine the kind of sRNA methylase that is responsible for the increase in extent of methylation. This is presented as specific activities (methyl groups per nucleotide molecule), although it should be understood that these figures do not represent the original specific activity of the product since carrier sRNA was added. However, the comparative aspects of the specific activities of nucleotides from the two preparations should be quite meaningful since the same amount of carrier sRNA was added to each preparation. The ethionine preparations as compared with the control liver enzymes have increases in the cytidylate, adenylate, and uridylate methylase activities as compared to guanylate methylase activity.

It is remarkable that in an analysis of sRNA by Tsutsui et al. (1966), using normal and neoplastic human mammary gland sRNA methylases that cytidylate, adenylate, and uridylate methylases were not detected in normal tissue but were clearly demonstrated using tumor extracts. In T_2 infected E. coli cells a two-fold increase in uridylate and adenylate methylation occurred relative to guanylate methylation when compared to noninfected bacterial activities (Wainfan et al., 1965).

It appears that after six months of ethionine feeding, a neoplastic

TABLE II

Specific Activities of Nucleotides from Methylated sRNA
($\mu\mu$moles methyl groups/μmoles of nucleotide)

Nucleotide	Control	Ethionine treated
CMP	1.5	35.2
AMP	2.2	134.7
GMP	407.3	349.0
UMP	47.6	74.4

Each reaction mixture contained: 500 μmoles of tris pH 7.6, 250 μmoles of reduced glutathione, 150 μmoles of $MgCl_2$, 5 mg of E. coli K12 sRNA, 2.5 μC of S-adenosyl-L-(C^{14} methyl)-methionine (55 mC/mmole), 4.7 and 3.7 ml of 100,000 x g supernatant liver cell fraction from control and ethionine fed mice respectively, in a total volume of 10 ml and incubated for 120 min at 37°C. 5 ml of 3 M hydroxylamine was then added and reaction mixtures were allowed to stand for 10 min at 27°C, followed by the addition of 50 mg of E. coli K12 sRNA. The phenol method of Kirby (1953) was used to isolate the final product which was subjected to alkaline hydrolysis and paper electrophoresis (8 hrs - 3000 volts, using an acetate buffer pH 4.6). The ultraviolet absorbing areas were eluted and ultraviolet absorption spectra were taken before measuring the radioactivity with a gas-flow counter of 37 per cent efficiency.

state exists in liver cells with respect to sRNA methylases. The increase in the extent of methylation of a given quantity of sRNA clearly demonstrates that sRNA methylases appear, after ethionine treatment, with capacities to methylate previously unavailable sites on the E. coli sRNA and presumably in vivo on mouse liver sRNA. This is indicated by the increase in extent of methylation in the reaction mixtures not containing E. coli sRNA. An analysis of the extent and kind of methylated nucleotides in mouse liver sRNA from ethionine fed mice should prove of great interest in light of the above findings. "Neomethylase" activity may effect sRNA function by altering secondary and tertiary structures or coding properties, and these changes may be basic to the mechanism by which ethionine induces a neoplasm.

ACKNOWLEDGMENTS

I wish to thank Mrs. P. McFarland for her excellent assistance with these experiments. This investigation was supported in part by an allocation from a Public Health Service Grant No. 1 R01 CA 10476-01 and Grant E-438 (Janice M. Blood Memorial Grant for cancer research from the American Cancer Society). The principles of laboratory animal care as promulgated by the National Society of Medical Research are observed in this Laboratory.

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